Exploration of Xanthone Derivatives as Anticancer Agents against Colorectal Cancer

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ABSTRAK

Kanker kolorektal saat ini menempati posisi ketiga terbanyak di dunia dan menjadi penyebab kematian kedua akibat kanker setelah kanker paru. Kemoterapi yang saat ini masih menjadi modalitas utama kanker kolorektal stadium I sampai III, penggunaannya dibatasi oleh tingginya resistensi dan risiko efek samping. Hal ini menyebabkan penggalian obat antikanker kolorektal terus dilakukan dan dikembangkan, termasuk pengembangan senyawa yang berasal dari alam. Xanthone menjadi salah satu senyawa dari alam yang berpotensi untuk dikembangkan sebagai antikanker pada kanker kolorektal, karena mempunyai aktivitas sitotoksik dan antiproliferatif yang baik. Mekanisme sitotoksik dan antiproliferatif senyawa xanthone secara *in vitro* terjadi melalui berbagai mekanisme yang melibatkan induksi apoptosis, penghambatan terhadap siklus sel, sedangkan secara *in vivo* mampu menurunkan ukuran tumor. Sebagaimana lazimnya penggunaan kombinasi pada kanker kolorektal, senyawa xanthone juga terbukti efektif untuk digunakan sebagai ko-kemoterapi terhadap obat kemoterapi standar yang saat ini ada. Tulisan ini bertujuan untuk memberikan gambaran mengenai kanker kolorektal, patologi, faktor risiko dan faktor protektif, sekaligus membahas terapi terkini dan potensi senyawa xanthone sebagai alternatif terapi yang dapat dikembangkan di kemudian hari untuk kanker kolorektal.

Kata kunci : kanker kolorektal; xanthone; apoptosis; siklus sel; efek sinergistik

ABSTRACT

Colorectal cancer currently occupies the third position of globally cancer morbidity and is the second leading cause of cancer death after lung cancer. Chemotherapy administration is still the main modality for the colorectal cancer stage I to III. Its usage is limited, since its high resistance and risk of side effects. Thus, exploration and development of novel colorectal anticancer drugs, including compounds derived from nature origin is needed. Xanthone becomes one natural compound which may be potentially developed as an anti-colorectal cancer due to its cytotoxic and anti-proliferative activities. The in vitro cytotoxic and antiproliferative actions of xanthone compounds occur through a variety of mechanisms involving both apoptotic induction and inhibition of cell cycle, while in vivo may reduce the tumor size. Since colorectal cancer is frequently treated in a combination therapy, xanthone compounds have also been studied in a combination use and proven effective as co-chemotherapy with the standard chemotherapy drugs. This paper aims at providing an overview of colorectal cancer, pathology, risk factors and protective factors, as well as discussing the current therapies and potential xanthone compounds as an alternative therapy which may be developed later for the colorectal cancer.

Keywords: colorectal cancer; xanthone derivatives; apoptosis; cell cycle; synergistic effect

Introduction

A cancer is a disorder in the form of abnormal cell growth and distribution deviating from their original behavior. These cells have unstable structure and are able to grow uncontrollably (Floor *et al.*, 2012). Population of cancer cells or its model, namely *cancer cell lines*, shows certain biochemical and biological characteristics, which may be equal or slightly different between cancer types. These special characteristics are often called *the hallmarks of cancer* (Hanahan & Weinberg, 2011). The whole characteristics are influenced by various oncogenes and tumor suppressor genes, which mostly have mutated and had genomic change. Such genomic change may be in the form of nucleotide addition, change in the number of copies of chromosomes or change in DNA (MacConaill & Garraway, 2010). These changes may occur because of external (environment) and internal (genetic) factors.

Cancer occurrences keep increasing globally throughout the world. The 2018 data of the International Agency for Research on Cancer (IARC) show there are 18.1 million new cancer cases, with mortality of 9.6 million cases. The highest numbers of new cancer cases are lung cancer (11.6%), breast cancer (11.6%), colorectal cancer (10.2%), prostate cancer (7.1%) and stomach cancer (5.7%). The most common causes of death are lung cancer (18.4%), colorectal cancer (9.2%), stomach cancer (8.2%), liver cancer (8.2%) and breast cancer (6.6%) (Bray *et al.*, 2018). In Indonesia, the most common causes of death are breast cancer (16.7%), cervical cancer (9.3%), lung cancer (8.6%), colorectal cancer (8.6%) and liver cancer (5.3%) (World Health Organization, 2019).

Increasing morbidity due to cancer is caused by aging and increasing population adopting cancer triggering habits/behaviors, such as smoking, low physical activities and high-calorie food consumption (Jemal *et al.*, 2010). In Europe, in 2008 cancer causes the death of 1.23 million patients (Luengo-Fernandez, Leal, Gray, & Sullivan, 2013), while in 2012 deaths due to cancer increases to 1.75 million patients (Hanly, Soerjomataram, & Sharp, 2015). The lost productivity cost due to cancer death in the United States in 2000 is 115.8 billion United States Dollars (Bradley *et al.*, 2008), and 75 billion Euros in Europe in 2008. This lost productivity cost is primarily caused by lung cancer (23%), breast cancer (9%) and colorectal cancer (8%) (Hanly *et al*., 2015). The relatively low success rate of chemotherapy because of side effects and resistance of cancer cells (Workman *et al.*, 2013) makes explorations of various compounds, both of nature origin or synthetic, as anticancer keep conducted.

Genes involved in cancer occurrence may be classified into two, namely proto-oncogene and tumor suppressor gene, which actually play an important role in cell normal control. Proto-oncogene plays an important role in cell proliferation, survival and cell spreading, but in cancer occurrence, proto-oncogene changes to oncogene. Proto-oncogene which is dominant phenotype in normal cells is strictly controlled to prevent excessive cell growth (Harrington, 2011). Supervisory function is played by tumor suppressor gene, which will stop cell growth so as not to be overgrown (Larson *et al.*, 2013). In certain condition, proto-oncogene may change to oncogene which trigger cancer occurrence. This imbalance between tumor suppressor gene and oncogene plays an important role in cancer occurrence, whether oncogene is dominant or even tumor suppressor gene malfunctions.

Colorectal Cancer: Pathology, Risk Factor and Protective Factor

Colorectal cancer is a cancer derived from abnormal cell growth in colon or rectum. It may be called colon cancer or rectal cancer, depending on which part they grow in. Nevertheless, the two are often classified into the same terminology as colorectal cancer since they have quite similar findings (American Cancer Society, 2017). Colorectal cancer is the most frequently occurring cancer in gastrointestinal system (Granados-Romero *et al.*, 2017).

Colorectal cancer is associated with mutation to oncogene, tumor suppressor gene or genes involved in DNA repair mechanism. A condition called genomic instability becomes the pathogenic base of colorectal cancer. The genomic instability is in the form of chromosomal instability (CIN), microsatellite instability (MSI) and CpG island methylator phenotype (CIMP) (Mármol *et al.*, 2017). These pathogenic pathways are marked with pathologic image, carcinogenesis mechanism and different tumorigenesis process (Mojarad *et al.*, 2013).

CIN pathway is deemed the classical pathogenic base of colorectal cancer occurrence, considering that 80-85% cases have mutation in this pathway. CIN genomic instability is marked with imbalance of the number of chromosomes resulting in aneuploidy condition and loss of heterozygosity (LOH). Some important genes disturbed in this pathway are, among others, Adenomatous Polyposis Coli (APC), KRAS, PI3K and P53. Mutation in APC causes movement of β-catenin to nucleus and stimulates tumorigenesis and invasion (Pino & Chung, 2010). Wnt signaling pathway/β-catenin is known to play a major role in colorectal cancer pathology, in which this pathway is a negative regulator in APC (Novellasdemunt, Antas, & Li, 2015). Mutation in KRAS and PI3K activates MAP kinase stimulating cell proliferation, while p53 mutation (in the form of loss of function) causes disturbance in the checkpoint function of cell cycle, thus cell keeps growing uncontrollably (Pino & Chung, 2010).

Microsatellite instability (MSI) mechanism involves loss in DNA repair mechanism, on mismatch repair (MMR) gene. This defect causes reduced ability of tumor cell to repair certain DNA chain, thus mutation occurs to the region (Mármol *et al.*, 2017). This MSI plays a role in 15-20% of all colorectal cancer cases. MSI induced tumorigenesis process is mediated through damage to MMR which plays an important role in maintaining cell genetic stability through DNA repair mechanism, repairing any error arising in gene, inhibiting recombinant between non-identical DNA order and repairing existing damage to DNA (Mojarad *et al.*, 2013).

The third pathway in colorectal cancer pathology is a condition called CpG island methylator phenotype (CIMP), which is hypermethylation on oncogene promoter of CpG island, resulting in gene silencing and lost expression of proteins in tumor suppressor gene (Gobbi *et al.*, 2010). CpG area is an area inside genome rich of dinucleotide of cytosine preceding guanine (CpG) existing in the part of promoter. In colorectal cancer, this area is massively hypermethylated, thus silencing the gene's expression. Although the cause of hypermethylation is not certainly known, but facts show that colorectal cancer and CIMP have different epidemiology, histology, precursor lesion and molecular picture (Mojarad *et al.*, 2013). Three conditions of genetic instability in colorectal cancer, namely chromosomal instability (CIN), microsatellite instability (MSI), and CpG island methylator phenotype (CIMP), show that colorectal cancer pathology involves genetic and epigenetic processes.

Some risk factors associated with colorectal cancer are hereditary, age, chronic inflammation on bowel, lifestyle, smoking and alcohol consumption factors (Mármol *et al.*, 2017). Hereditary factor contributes to 35% cases of colorectal cancer. Some histories in family, which are risk factors are hereditary diseases such as familial adenomatous polyposis and Lynch syndrome. Some other hereditary conditions are ulcerative colitis history, Crohn's disease, polyp on colon, rectum, ovary, endometrium, breast cancer and diabetes mellitus histories. These disorders are associated with increased risk of experiencing colorectal cancer up to 30-50% (Granados-Romero *et al.*, 2017). Adenomatous polyp often occurs to adult above 50 years old, but not all will be malignant.

Colon polyp which is at risk of becoming colorectal cancer is one in a number of more than twenty, more than 1 cm in size, with dysplasia found on polyp (American Cancer Society, 2008), especially one located in right colon, and with family history of colorectal cancer (Granados-Romero *et al.*, 2017).

Age is a risk factors of colorectal cancer. Adults above 50 years old are more likely to have the disease. Inflammatory process in bowel also increases 3.7% the risk of having colorectal cancer, while Crohn's disease increases the risk for 2.5%. The reason is that chronic inflammation triggers dysplasia of colorectal cells, and the possibility to have anaplasia developing to cancer is higher (Mármol *et al.*, 2017).

Lifestyle is deemed to be the factor to contribute to colorectal cancer occurrence, particularly related to sedentary lifestyle. This sedentary lifestyle is associated with obesity, involving imbalance between food intake and high visceral adipose tissue (VAT), namely total body fat component which is hormonally active. This fat component actively produces pro-inflammatory cytokine which stimulates inflammatory condition in colon and rectum, insulin resistance and metabolic enzyme modulation, such as adiponectin and leptin. Food also contributes to colorectal cancer occurrence up to 70%, such as red meat consumption (releasing carcinogenic heme group), grilled meat (producing heterocyclic amines and polycyclic hydrocarbons) and alcohol consumption (producing side product acetaldehyde). Meanwhile, smoking increases colorectal cancer risk up to 10.8%, since its carcinogenic metabolic content (for example, nicotine) easily reaches bowel and induces polyp occurrence (Mármol *et al.*, 2017).

Colorectal cancer is known to have Cyclooxygenase-2/COX-2 enzyme and epidermal growth factor (EGF) overexpression. COX-2 overexpression contributes to activator protein-1 (AP1) activity which will bind to EGF receptor (EGFR), triggering transcription. Administration of COX-2 enzyme inhibiting protective agent may inhibit colorectal cancer growth, since it is able to reduce EGF and COX-2 overexpression, which increases at 80% cases of early colorectal tumor (precancerous lesion). The other protective factor is high fiber diet, particularly fruits and vegetables (Granados-Romero *et al.*, 2017).

Colorectal Cancer Therapy

Therapy for colorectal cancer stadium 0 is in the form of tumor mass removal with colonoscopy, while for stadiums I, II and III is in the form of radical colectomy on the affected segment with a limit of more than 5 cm, along with lymphadenectomy of surrounding area. For colorectal cancer with distant metastasis, combination chemotherapy of Irinotecan with 5-Fluorouracyl (5-FU) and Leucovorin (IFL) needs to be conducted. This chemotherapy combination (IFL) evidently able to lengthen patient's survival at stadium III and IV up to 6-8 months, and improve their quality of life (Granados-Romero *et al.*, 2017).

Although chemotherapy remains the main modality in colorectal cancer therapy for the last decades, but its success rate is still low because of side effect and cancer cell resistance resulting from anticancer non-selective and non-specific working characteristics. Therefore, currently the development of anticancer is directed to explore anticancer which works selectively and specifically, directed to genomic and molecular abnormality (Workman *et al.*, 2013), which is called targeted therapy. In targeted therapy, drugs will work specifically on a certain part or pathology of cancer cells, but not on normal cells. Therefore, side effects may be avoided. This underlies various researches, which leads to compounds which are able to work specifically on cancer cells.

One of the targeted therapies used on colorectal cancer is vascular endothelial growth factor (VEGF) inhibitor, such as Bevacizumab, has been approved by the Food and Drug Association (FDA) in the United States. This drug works by inhibiting angiogenesis process in colorectal cancer. Addition of Bevacizumab to IFL combination causes synergic effect Bevacizumab will reduce cancer cell vascularization (including neovascularization). Thus, IFL drug distribution in cancer cells gets better (Granados-Romero *et al.*, 2017), as marked with improved outcome as presented in Table 1 below. Currently, alternatives are continuously sought from compounds which may potentially be targeted therapy for colorectal cancer.

Table 1. Comparison of colorectal cancer outcome with application of IFL combination with Bevacizumab addition to IFL combination

Parameter	IFL Combination	IFL Combination and Bevacizumab
Progression-free survival (month)	6.2	10.6
Overall response rate (%)	34.8	44.8
Average response duration (month)	7.1	10.4

Source : (Granados-Romero *et al.*, 2017)

Xanthone Potential as Alternative Anticancer for Colorectal Cancer

Xanthone compound is a group of heterocyclic compounds with heterocyclic main frame structure dibenzo-γ-pyrone. This structure is basic active compound, which is planar tricyclic frame with 1 pyran ring fused with 2 phenyl rings on its two sides (Yang *et al.*, 2014) (Figure 1). Xanthone derivative compound is one of the relatively promising compounds to be developed as an alternative anticancer. Xanthone is a natural phenolic compound long known as antioxidant because of its activities as metal chelator, free radical suppressor and lipid

peroxidation inhibitor (Pinto *et al.*, 2005). One of the plants containing xanthone is *Garcinia mangostana (G. mangostana)*, with xanthone compounds in the form of α-mangostin and γ-mangostin, which are known to have antiproliferative effect on colorectal cancer cell line (Yoo *et al.*, 2011) (Figure 2).

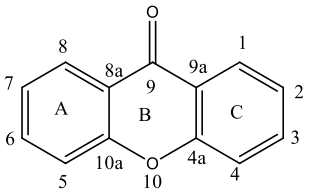
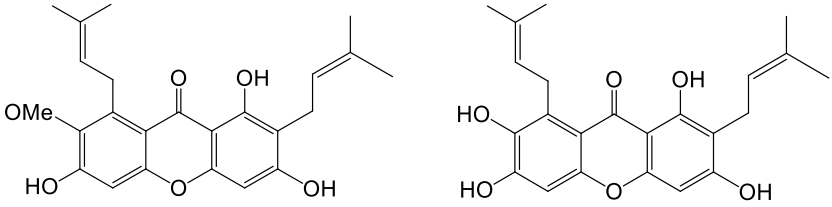


Figure 1. Xanthone compound structure



(A) α-mangostin (B) γ-mangostin

Figure 2. Structure of xanthones derived from *G. mangostana* plant.
(A) α-mangostin, (B) γ-mangostin

In the last decade, many researches have been conducted showing that xanthone compounds of natural origin evidently have effect on improvement of apoptosis and inhibition of cell cycle, by triggering various caspase enzymes (Kuete *et al.*, 2014), increase in Bax protein, inhibition of Bcl-2 and nuclear factor kappa B/NF-κB (Mohan *et al.*, 2012) and inhibition of various cyclins (Kuete *et al.*, 2014). Other researches show that apoptosis and cell cycle disorders resulting from deregulation of p53, Bcl-2, NF-κB and various caspases are related to cyclooxygenase-2 (COX-2), vascular endothelial growth factor (VEGF) and telomerase functions disorder (Low & Tergaonkar, 2013) .

The mostly studied isolated natural xanthone compound is α-mangostin, although some other xanthones have also been studied. α-mangostin compound is isolated from mangostin peel (*G. mangostana*), which has been explored for a long time and evidently has cytotoxic activity on various types of cancer cells, both in vitro and in vivo (Ibrahim *et al.*, 2016). Many previous studies prove how this natural xanthone compound influences colorectal cancer cells. Ethanol extract of α-mangostin derived from *G. mangostana* is able to inhibit the growth of colorectal cancer cell HCT 116 with cytotoxic activity (the maximal half inhibitory concentration/IC_{50}) of 6.5–9.2μg/mL (Aisha *et al.*, 2011). Matsumoto *et al*. (2005) conduct a research on some natural xanthone compounds and find that xanthone compound with substitution of hydroxy and methoxy groups in different position on side chain of xanthone frame has cytotoxic and antiproliferative activity on colon cancer line cell DLD-1, with IC_{50} value between 5–20μM. Nakagawa *et al.* (2007) also find the same, in which the xytotoxic activity of xanthone compound on cancer line cell DLD-1 is with IC_{50} value 7.5μM. Another research conducted by Han *et al.* (2009) finds that on different colorectal cancer line cell, namely HT29, it shows this cytotoxic activity between 1.7–9.1μM. On different colorectal cancer line cell, namely COLO 205, α-mangostin compound shows cytotoxic activity at concentration 9.74μg/mL (Watanapokasin *et al.*, 2011). Other research shows that cytotoxic activity on line cell SW480 is achieved at concentration 15μM (Yoo *et al.*, 2011). On other colorectal cancer line cells, namely CX-1, MIP-101 and SW620, xanthone compound has cytotoxic activities with IC_{50} values respectively 17.7; 10.0; and 16.1 μg/mL (Watanapokasin *et al.*, 2010). Therefore, it is clear that xanthone compound's cytotoxic activity on colorectal cancer cells are classified as strong, since it is under 10μg/mL or under 20 μM.

Watanapokasin *et al.* (2010) find that xanthone compound has cytotoxic and antiproliferative activity on colorectal cancer line cell COLO 205 in vitro and in vivo in mouse. Cytotoxic and antiproliferative activity in vitro occurs because of apoptosis induction through caspase–3 and –8 activation and cytochrome c release. An in vivo study on mouse induced with cell COLO 205 subcutaneously, xanthone administration at low dose (0.024; 0.12; and 0.6mg per tumor) is able to inhibit tumor growth. Xanthone administration at higher dose, 3.0mg per tumor, causes tumor size to shrink and even disappear in some mice (Watanapokasin *et al.*, 2010). α-mangostin extract is also able to inhibit cancer growth induced with cancer cell HCT 116 subcutaneously on mice. α-mangostin administration causes significantly shrunk size of tumor on day 15 at concentration 0.5%b/b and day 20 at concentration 0.25b/b. Even in sub-cytotoxic concentration, α-mangostin administration is able to inhibit three phases in tumor metastasis process, namely cell migration, cell invasion and clonogenicity. This practically implies xanthone compound as anti-metastasis agent (Aisha *et al.*, 2011; Aisha *et al.*, 2012).

α-mangostin compound is able to induce apoptosis through stimulation of caspase–3, –7, –8 and –9, causing DNA fragmentation, chromatin condensation and lost potential of mitochondrial membrane (Aisha *et al.*, 2011; Watanapokasin *et al.*, 2011). α-mangostin compound causes up regulation of signaling pathways MAPK/ERK, c-Myc/Max, and p53 (Aisha *et al.*, 2012) and also release of cytochrome c, p53, Bax and Bmf (Watanapokasin *et al.,* 2010 ; Watanapokasin *et al.*, 2011), in which all of these signaling pathways cause increase in apoptosis. Apoptosis induction through intrinsic pathway (mitochondria) also occurs because of down regulation on signaling pathways involving MAPK kinase and serine/threonine kinase Akt (Matsumoto *et al.*, 2005). Other than caspase activation, apoptosis mechanism of xanthone compound may also occur without involving caspase (caspase-independent) through endonuclease-G release. Endonuclease-G release is a nuclease existing in mitochondria, and able to induce nucleosomal DNA fragmentation. This is evident with the increased level of endonuclease-G a moment after α-mangostin administration, in addition to increase in cytochrome c and AIF (Nakagawa *et al.*, 2007), both will increase the permeability of mitochondrial membrane, thus apoptosis occurs.

Cytotoxic and antiproliferative mechanism of xanthone compound occurs through inhibition of cell cycle involving cyclin, cdc2 and p27, thus cell is suspended in G_1 and S phases (Matsumoto *et al.*, 2005). Inhibition on CDK4 also contributes to controlling G1 checkpoint, as determiner of cell which will enter S phase. Inhibition on CDK4 will cause cell cycle suspended in G1 and does not enter S phase (Vemu *et al.*, 2019). Xanthone compound's antiproliferative activity also occurs through inhibition of Wnt/β-catenin signaling, contributing greatly to colorectal cancer carcinogenesis. Xanthone compound administration for 48 hours causes decrease in TCF/β-catenin transcription activity which is directly proportional to dose increase, as well as reduce mRNA and β-catenin protein expression (Yoo *et al.*, 2011). In most of colorectal cancer cases, there is excessive activation of this signaling pathway, thus currently one strategy of colorectal cancer therapy is directed to inhibiting such signaling pathway (Novellasdemunt *et al.*, 2015).

As already known, colorectal cancer therapy is commonly conducted in combination, instead of single application. Xanthone compound has also been tested as chemotherapy agent in combination on colorectal cancer line cell DLD-1, in combination on 5-FU. Simultaneous use of α-mangostin with 5-FU at concentration each 2.5μM shows synergistic effect in inhibiting cancer cell proliferation, compared to separate use of α-mangostin and 5-FU at concentration 5μM in single application (Nakagawa *et al.*, 2007). This conforms to the theory of synergy of anticancer drug, in which simultaneous use of two types of drugs at certain concentration or dose present equal effect to or higher effect than separate use of the two drugs at the same dose or concentration (Tsakalozou, Eckman, & Bae, 2012).

Besides for therapy, xanthone compound also serves as chemoprevention in colorectal cancer. The study conducted by Nabandith, *et al.* (2004) shows that α-mangostin administration in diet is able to inhibit the progressivity of rat's precancerous colon lesion induced with 1,2-dimethylhydrazine (DMH) 40mg/kgBW (once a week for 2 weeks) subcutaneously. α-mangostin administration is able to prevent dysplastic foci and β-catenin accumulation on colonic crypt and also reduce proliferating cell nuclear antigen (PCNA) index which is the signifier of actively dividing cell. Therefore, α-mangostin evidently serves as chemoprevention in colorectal cancer occurrence, including in preventing precancerous lesion development to cancer. Table 2 below summarizes some cytotoxic and antiproliferative activities of xanthone derivative compounds studied. The apoptosis induction and colorectal cancer proliferation inhibition mechanism by xanthone compounds may be observed in Figure 3.

Table 2. Cytotoxic and antiproliferative activities of xanthone derivative compounds and their mechanism

Cancer line cell	IC_{50} value	Action mechanism	Reference
Precancerous lesion	-	As potent chemoprevention against colorectal cancer carcinogenesis on rats, by reducing dysplastic foci, β-catenin accumulation on crypt and reducing PCNA	(Nabandith *et al.*, 2004)
DLD-1	5–20 μM	Inhibit cyclin, cdc2 and p27 of cell cycle and induce apoptosis through down regulation of MAPK kinase and serine/threonine kinase Akt in colorectal cancer line cell DLD-1	(Matsumoto *et al.*, 2005)
DLD-1	7.5 μM	Induce apoptosis which does not depend on caspase, by releasing endonuclease-G from mitochondria and increasing miRNA-143 expression on cancer line cell DLD-1	(Nakagawa *et al.*, 2007)
SW480	15 μM	Inhibit colorectal cancer cell proliferation through β-catenin gene regulation on Wnt/β-catenin signaling pathway	(Yoo *et al.*, 2011)
COLO 205, CX-1, MIP-101, SW620	7.50–17.7 μg/mL	Induce apoptosis through activation of caspase–3, –8 and cytochrome c in vitro, and in vivo reduce cancer progressivity and kill cancer cells	(Watanapokasin *et al.*, 2010)
COLO 205, MIP-101,	9.74–19.6 μg/mL	Induce apoptosis through activation of caspase–3, –8, –9, cytochrome c, p53, Fas, Bid, also Bax and Bmf (famili Bcl-2	(Watanapokasin *et* al., 2011)

SW620		pro-apoptosis)	
HCT 116	6.5 µg/mL	Induce apoptosis in vitro through activation of caspase–3, –7, –8 and –9 on cancer line cell HCT 116, inhibit cell migration and invasion process and clonogenicity of cell HCT 116 and reduce tumor size in vivo on rats induced with cell HCT 116 subcutaneously	(Aisha *et al.*, 2012)
HCT 116	12.1 µM	Inhibit cell cycle through inhibition on CDK4/cyclin D1	(Vemu *et al.*, 2019)
HT 29	1.7-9.1 µM	Mechanism not tested	(Han *et al.*, 2009)

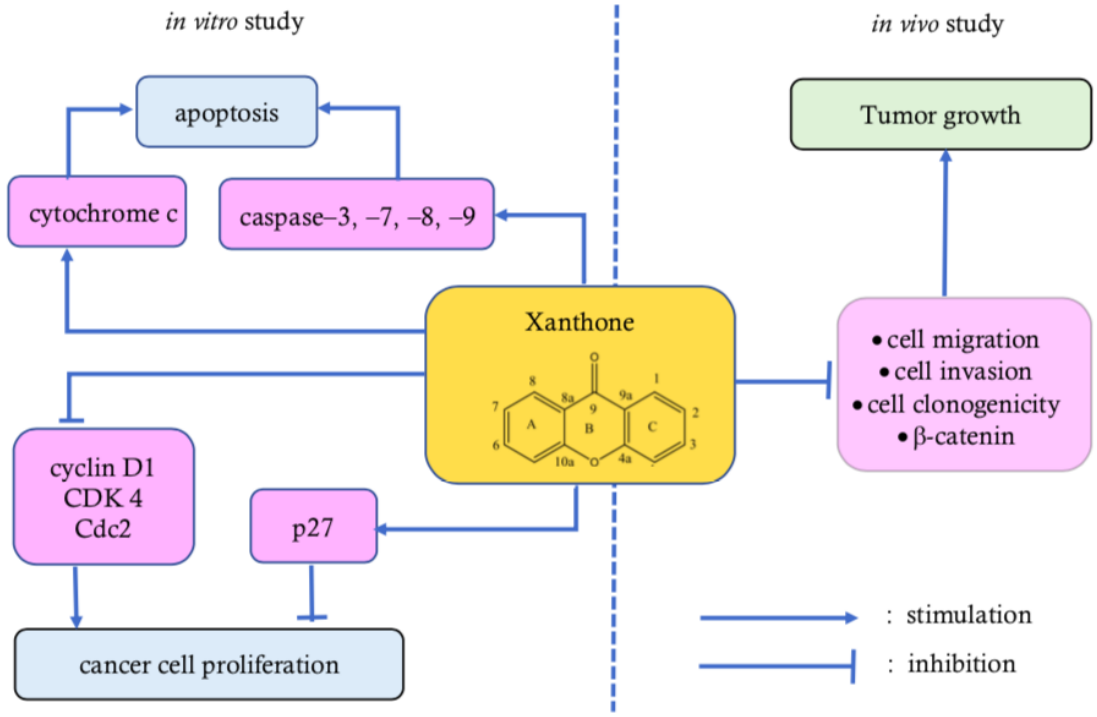


Figure 3. Apoptosis induction and colorectal cancer proliferation inhibition mechanism by xanthone compounds

Current technology development in drug delivery has led to the use of nanoparticle preparation in the form of drug preparation. Study shows that phenolic compounds in fruits and plants have advantage when it is packed in preparation using nanocarrier, allowing drug to achieve its target better. This is possible since nano-preparation increases drug bioavailability by increasing drug solubility, protecting drug against enzymatic hydrolysis, increasing specific drop surface area by regulating drop size up to nanometer and increasing drug permeability induced by surfactant (Abdelbary, Amin, & Salah, 2013). Nanoparticle formulation is also able to reduce risk of drug degradation by reducing drug intra-enterocyte metabolism and stimulating drug transportation through intestinal lymphatic system to prevent drug from getting metabolized in the liver (Cherniakov, Domb, & Hoffman, 2015). Aisha *et al.* (2015) have made xanthone compound in the form of nanoparticle preparation with nanoprecipitation method using Eudragit RL100 and Eudragit RS100. Test on colorectal cancer line cell HCT 116 proves that the preparation has high solubility, which even increases to 1.250µg/mL. Besides, the endocytic uptake of cationic nanoparticle is also higher, allowing better xanthone intracellular delivery process and reducing colorectal cancer cell resistance risk. Further research certainly needs to be conducted related to xanthone compound cytotoxic activity in the form of this nanoparticle, in order to prove the effectiveness of in vivo drug delivery of the nano-preparation.

Conclusion

Alternative therapies for colorectal cancer are continuously conducted, considering that current standard chemotherapy is limited with the risks of side effects and high resistance. Xanthone compound as one alternative of natural compounds developed as anticolorectal cancer shows good cytotoxic and antiproliferative activities, with IC_{50} value under 10µg/mL or under 20µM. Various researches show that xanthone compound is able to induce apoptosis, either through caspase activation or without through caspase (*caspase-independent*), inhibit cell

cycle through cyclin and CDK4 inhibition and also inhibit Wnt/β-catenin signaling pathway with important role in colorectal cancer carcinogenesis. Besides for therapy, xanthone compound is also effective for colorectal cancer chemoprevention by inhibiting the progressivity of precancerous colon lesion to develop to cancer. However, further research is necessary to ensure the effectiveness and to explore the action mechanism of this xanthone compound *in vivo* for certain, which *in vitro* has been frequently proven on various different colorectal cancer line cells.

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